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14. ABSTRACT

Lower body negative pressure (LBNP), a model of hemorrhage (Hem), shifts blood volume to the legs and elicits central hypovolemia. This study compared the effects of LBNP to actual Hem. Baboons (n=14) were instrumented to measure pulse pressure (PP), central venous pressure (CVP), and stroke volume (SV). Blood was removed in four steps: 6.25%,12.5%, 18.75%, and 25% of total blood volume. Four weeks after Hem, the same animals were subjected to four levels of LBNP which changed PP and CVP as seen during Hem. Blood sampled at baseline and maximum Hem or LBNP measured blood gases, hematocrit, hemoglobin, plasma renin activity (PRA), vasopressin (AVP), epinephrine (EPI) and norepinephrine (NE). Hemodynamic responses to Hem and LBNP were identical. Hem decreased hematocrit, hemoglobin, and central venous oxygen saturation (ScvO2). In contrast, LBNP increased hematocrit, and hemoglobin, while ScvO2 remained unchanged. Hem caused greater (pâ¤0.01) elevations in AVP and NE than LBNP, while PRA and EPI did not differ between studies. Thus, stepwise Hem elicited hemodynamic changes which could be mimicked with distinct levels of LBNP, but loss of red blood cells during Hem may result in greater neurohumoral activation. We conclude that while LBNP does not elicit the same effect on blood cell loss as Hem, LBNP mimics the integrative cardiovascular response to Hem, and validates the use of LBNP as an experimental model of Hem.

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Validation of lower body negative pressure as an experimental model of hemorrhage

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Hinojosa-Laborde C, Shade RE, Muniz GW, Bauer C, Goei KA, Pidcoke HF, Chung KK, Cap AP, Convertino VA. Validation of lower body negative pressure as an experimental model of hemorrhage. *J Appl Physiol* 116: 406–415, 2014. First published December 19, 2013; doi:10.1152/japplphysiol.00640.2013.—Lower body negative pressure (LBNP), a model of hemorrhage (Hem), shifts blood to the legs and elicits central hypovolemia. This study compared responses to LBNP and actual Hem in sedated baboons. Arterial pressure, pulse pressure (PP), central venous pressure (CVP), heart rate, stroke volume (SV), and $+dP/dt$ were measured. Hem steps were 6.25%, 12.5%, 18.75%, and 25% of total estimated blood volume. Shed blood was returned, and 4 wk after Hem, the same animals were subjected to four LBNP levels which elicited equivalent changes in PP and CVP observed during Hem. Blood gases, hematocrit (Hct), hemoglobin (Hb), plasma renin activity (PRA), vasopressin (AVP), epinephrine (EPI), and norepinephrine (NE) were measured at baseline and maximum Hem or LBNP. LBNP levels matched with 6.25%, 12.5%, 18.75%, and 25% hemorrhage were -22 ± 6, -41 ± 7, -54 ± 10, and -71 ± 7 mmHg, respectively (mean ± SD). Hemodynamic responses to Hem and LBNP were similar. SV decreased linearly such that 25% Hem and matching LBNP caused a 50% reduction in SV. Hem caused a decrease in Hct, Hb, and central venous oxygen saturation ($ScvO_2$). In contrast, LBNP increased Hct and Hb, while $ScvO_2$ remained unchanged. Hem caused greater elevations in AVP and NE than LBNP, while PRA, EPI, and other hematologic indexes did not differ between studies. These results indicate that while LBNP does not elicit the same effect on blood cell loss as Hem, LBNP mimics the integrative cardiovascular response to Hem, and validates the use of LBNP as an experimental model of central hypovolemia associated with Hem.

blood loss; central hypovolemia; stroke volume; blood pressure; central venous pressure; cardiac output

HEMORRHAGE IS THE PRIMARY cause of death within the first hour of injury in military and civilian trauma patients (5, 50). Early detection of central hypovolemia in bleeding patients is critical to early life saving intervention (15). Therefore it is important to develop a valid model for understanding the physiology of human hemorrhage especially during the early stages of hypovolemia. Lower body negative pressure (LBNP) has been used in our laboratory as a highly reproducible experimental model of hemorrhage to investigate the physiological responses to hypovolemia (7). LBNP causes a reduction in pressure surrounding the lower extremities. As pressure is progressively lowered, blood volume shifts from the thoracic circulation to the lower extremity circulation and creates a state of central hypovolemia. Stepwise decreases in LBNP are applied to a maximum of -100 mmHg or until the subject experiences signs of presyncope. Correlations between LBNP and blood loss volumes have been estimated by comparing hemodynamic changes during LBNP and hemorrhage in two or more study populations, but there are no direct measurements of actual blood volume loss matched with multiple LBNP levels (12).

In addition to the hemodynamic effects, hemorrhage adversely affects multiple body systems and can lead to neuroendocrine and metabolic derangements (53). Specifically, hypovolemia and hypoperfusion can activate the release of vasoactive hormones to maintain perfusion of high-priority organs like the heart and brain at the expense of perfusion to the gut, skin and other "nonvital" end organs (12, 53). Increased cardiac contractility, a compensatory mechanism that temporarily increases stroke volume, is also stimulated by hypovolemia and the release of catecholamines (2). Fluid shifts can produce electrolyte abnormalities and changes in osmolarity, while hypoperfusion leading to global dysoxia can result in elevated lactate levels (37). Although the LBNP procedure qualitatively causes hemodynamic, neurohumoral, and metabolic responses similar to hemorrhage (7, 12, 31, 57), a direct comparison of these responses with multiple levels of actual hemorrhage has not been reported.

Since results from LBNP experiments have provided new insight into mechanisms underlying hemodynamic decompensation during hemorrhage in humans (9, 45, 47), and subsequently information critical to the development of advanced clinical decision support monitoring, we proposed a study to determine the validity of LBNP as a model of hemorrhage. The purpose of this study was to test the hypothesis that the hemodynamic, neurohumoral, and metabolic responses associated with blood pressure regulation during specified levels of LBNP are equivalent to multiple levels of blood loss using a nonhuman primate model.

MATERIALS AND METHODS

Animals. Adult male baboons ($n = 14$) were housed in individual cages in association with other baboons beginning 2 wk prior to experiments and ending 1 wk after study termination. The baboons were fed twice a day and given water ad libitum. Experiments were started when the baboons were accustomed to laboratory conditions. The protocol was submitted to and approved by the Institutional Animal Care and Use Committee of the Texas Biomedical Research Institute, San Antonio, TX. The study was conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulations, and the principles of the *Guide for the Care and Use of Laboratory Animals*.

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Blood volume determination. Blood volume was determined in a separate group of male baboons ($n = 9$) to prevent the need for an additional surgery and sedation procedure in animals exposed to hemorrhage and LBNP. Blood volume was calculated from the measurements of plasma volume with Evans blue dye technique (51) and hematocrit. Arterial blood samples were obtained before and 5 min after a bolus venous injection of Evans blue dye (Sigma) solution (0.5% in sterile saline, 1 ml/kg). Blood samples were analyzed to determine hematocrit by centrifugation, and plasma concentration of Evans blue by spectrophotometer compared with a standard curve.

Surgical procedures. On the day of each experimental session (hemorrhage or LBNP), the baboons were sedated with ketamine (10 mg/kg im). Sedation was maintained during the experiment with ketamine (10 $mg \cdot kg^{-1} \cdot h^{-1}$ iv) and valium (0.1 $mg \cdot kg^{-1} \cdot h^{-1}$ iv). The animals were intubated to maintain an open airway and allowed to breathe spontaneously during the experiments. For the hemorrhage and LBNP experiments, two vascular catheters were inserted using aseptic technique via small skin incisions to visualize the vessels. Arterial pressure and pulse pressure were measured via a pressure transducer attached to a Millar solid-state catheter inserted in the axillary artery. Central venous pressure (CVP) was measured via a pressure transducer attached to a fluid-filled catheter inserted in the axillary vein and advanced into the vena cava until the tip of the catheter was located proximal to the right atrium. Catheter tip placement was confirmed by assessing venous pressure waveforms for characteristics consistent with CVP. For hemorrhage experiments only, two additional catheters were inserted in the femoral artery and vein for blood removal and replacement, respectively. After catheter placement, ECG leads were attached to monitor heart rate. At the completion of the hemorrhage and LBNP experiments, the catheters were removed and the skin incisions at the catheter insertion sites were sutured closed.

Hemorrhage experiment. Blood pressure, heart rate, and CVP were recorded continuously during the experiment. Baseline values were monitored for 20 min prior to a stepwise hemorrhage of 25% blood volume calculated based on measurements of blood volume in a different group of baboons. The four steps of hemorrhage represented 6.25%, 12.5%, 18.75%, and 25% blood volume. Blood was removed by reversed infusion pump (50 ml/min) via the femoral artery, and each hemorrhage step was held for 7 min. Shed blood was collected into sterile citrate phosphate dextrose blood donation bags. Systolic arterial pressure was monitored closely during the hemorrhage procedure, and a value lower than 70 mmHg was considered an indicator of impending cardiovascular collapse. As an a priori protocol termination criterion, the hemorrhage procedure was stopped prematurely if this systolic arterial pressure threshold was attained prior to 25% blood loss. After the last step of hemorrhage, shed blood was replaced by infusion pump (50 ml/min) via the femoral vein. Hemodynamic variables were allowed to stabilize for 20 min prior to removing the catheters. Upon recovering from sedation, the animals were returned to their home cages and monitored for the resumption of normal feeding and drinking behavior.

Lower body negative pressure experiment (LBNP). Four weeks after the hemorrhage experiment, the baboons were again sedated and instrumented with axillary artery and vein catheters and ECG leads. The animals were placed supine in an airtight LBNP chamber sealed at the level of the iliac crest by a neoprene skirt. Blood pressure, heart rate, and CVP were recorded continuously during the experiment. Baseline values were monitored for 20 min prior to a stepwise LBNP procedure that was designed to match pulse pressure and CVP during the animal's previous hemorrhage study. At the end of the procedure, the negative pressure was released, and hemodynamic variables were allowed to stabilize for 20 min. Catheters were removed, and the animals were allowed to recover from the effects of sedation before returning to their home cages.

Hematologic, blood gas, and chemical analyses. Bloods samples were collected from all animals ($n = 14$). However, due to technical difficulties during collection and processing of samples from 2 animals, blood analysis was conducted in 12 animals. Hemoglobin (Hgb) and hematocrit (Hct) were measured in EDTA venous blood samples (2 ml) at baseline and maximum (MAX) hemorrhage and LBNP time points with a hematology analyzer (Beckman Coulter, Brea CA). Venous blood (2 ml) collected in heparin at the same time points underwent blood gas analysis to determine pH, the partial pressure of oxygen (P_{O_2}) and carbon dioxide (P_{CO_2}), bicarbonate (HCO_3), base excess (BE), lactate, and central venous oxygen saturation ($ScvO_2$; CG4+iSTAT system, Abbott Point of Care). Blood chemistry analysis was conducted on additional venous blood samples (2 ml) for measurement of blood urea nitrogen (BUN), glucose, and sodium (Chem8 iStat System, Abbott Point of Care). Osmolarity (OSM) was calculated from the measured sodium (Na), glucose, and BUN parameters using the formula: $OSM = (1.86 \cdot Na^{+}) + (glucose/18) + (BUN/2.8)$. Samples were processed immediately upon collection.

Plasma renin activity, vasopressin, and catecholamine assays. Venous blood samples ($n = 12$, 6 ml/sample) were analyzed at baseline and MAX hemorrhage and LBNP time points. Blood samples were collected in EDTA tubes with reduced glutathione to minimize catecholamine oxidative degradation. The samples were immediately placed on wet ice and centrifuged at 4°C. Plasma samples were stored at −80°C until they were assayed.

Plasma renin activity (PRA) was calculated from the measurement of angiotensin I by radioimmunoassay [GammaCoat Plasma Renin Activity (CA-1553), DiaSorin]. The standard curve was fitted with a four-parameter logistic by nonlinear regression analysis (Sigmaplot) for the calculation of plasma angiotensin I concentration in hot (37°C) and cold (4°C) incubated aliquots of plasma. The radioimmunoassay results were used to calculate PRA (ng of angiotensin I generated per hour of incubation).

Plasma arginine vasopressin (AVP) was measured by radioimmunoassay after extraction using C18 cartridges [Ultrasensitive-ADH/AVP (RK-AR1), ALPCO Immunoassays]. The AVP standard curve was fitted with a four-parameter logistic by nonlinear regression analysis (Sigmaplot) for the calculation of plasma AVP concentration.

Plasma NE and EPI concentrations were measured by ELISA [BA E-5400 2-CAT (EPI/NE) Research ELISA, Rocky Mountain Diagnostics]. The ELISA measurements were made on a Thermo Labsystems plate reader (iEMS Reader MF). The NE and EPI standard curves were fitted with a four-parameter logistic by nonlinear regression analysis (Sigmaplot) for the calculation of plasma NE and EPI concentrations.

Data analysis. Continuous ECG, CVP, and arterial blood pressure waveform recordings were sampled at 500 Hz using LabView data-acquisition software during hemorrhage and LBNP experiments. Hemodynamic variables were analyzed during the last 3 min of each hemorrhage and LBNP step using a commercially available software program (WinCPRS, Absolute Aliens, Turku, Finland). Beat-to-beat stroke volume (SV) was derived from the arterial pressure waveform using the pulse contour method (32). Cardiac output was calculated as the product of SV and heart rate. Statistical analysis was conducted with commercially available software (SigmaStat; Systat Software). Vascular resistance was calculated as (diastolic pressure + 1/3 pulse pressure)/(SV × heart rate). Cardiac function was assessed from the arterial pressure waveform as the rate of change of pressure by units of time (the slope of the arterial pressure waveform, $+dP/dt$) (55). Two-way repeated-measures ANOVA was used to determine significant ($P \leq 0.05$) time effects during hypovolemia with hemorrhage or LBNP, and group effects (hemorrhage vs. LBNP) in hemodynamic responses. Data are expressed as means ± SD.

RESULTS

Hemorrhage volumes. Blood volume in a separate group of male baboons ($n = 9$) weighing 25.8 ± 2.7 kg was calculated to be 71 ± 8 ml/kg. Based on this measurement, hemorrhage volumes were determined in baboons that were used for hem-

Table 1. *Blood volume loss and matching LBNP levels*

	$n = 14$	$n = 14$	$n = 14$	$n = 12$
BV, %	6.25	12.5	18.75	24.5 ± 0.8
BV, ml	136 ± 13	271 ± 26	408 ± 39	529 ± 58
BV, ml/kg	4.5 ± 0.1	9.1 ± 0.2	13.6 ± 0.4	17.8 ± 0.6
LBNP, mmHg	−22 ± 6	−41 ± 7	−54 ± 10	−71 ± 7

Data are means ± SD. Blood volume (BV) loss values are represented as percent, absolute, and relative measurements. Lower body negative pressure (LBNP) levels that matched the BV loss are shown for each level of hemorrhage.

orrhage and LBNP experiments ($n = 14$, 30.6 ± 2.9 kg body wt). Blood loss volumes at 6.25%, 12.5%, 18.75% and 25% hemorrhage are shown in Table 1. All animals ($n = 14$) were hemorrhaged to a loss of 18.75% blood volume. However, the hemorrhage and matching LBNP procedures were terminated at this level of blood loss in two baboons who met the termination criterion prior to the last step of hemorrhage. The remaining 12 animals completed 23–25% (24.5 ± 0.8%) blood volume loss and matching LBNP.

Cardiovascular responses. Stepwise hemorrhage caused progressive decreases in CVP and pulse pressure (Fig. 1). Since CVP and pulse pressure were the matching variables which determined the levels of LBNP, the similarity in responses demonstrates the accuracy by which CVP and pulse pressure values were matched to hemorrhage during the LBNP experiment. Other hemodynamic responses to hemorrhage and LBNP are shown in Fig. 2. Average systolic arterial pressure, heart rate, SV, and cardiac output, vascular resistance, and $+dP/dt$ at baseline and during hemorrhage were not statistically distinguishable from those responses recorded during LBNP. All hemodynamic parameters, except vascular resistance, changed significantly during hemorrhage or LBNP. The levels of LBNP which corresponded with the four hemorrhage levels are shown in Table 1 and Fig. 2.

Pulse contour estimates of change in SV are more accurate than pulse contour estimates of absolute values of SV (32). Figure 3 shows the linear relationship between the percent change in SV from baseline during hemorrhage (slope = 2.1) and LBNP (slope = 2.2). This relationship revealed that 25% hemorrhage and the corresponding LBNP level resulted in a 50% and 53% reduction in SV, respectively.

Hematologic responses. MAX hemorrhage caused Hgb, Hct, and $ScvO_2$ to decline from baseline levels ($P < 0.001$). In contrast, MAX LBNP caused an increase in Hct and Hgb ($P < 0.001$), and did not affect $ScvO_2$ (Fig. 4, *A, B*, and *F*). MAX hemorrhage and LBNP had no effect on HCO_3, BE, and lactate (Fig. 4, *C–E*).

Other indexes of metabolic function and electrolyte balance are shown in Table 2. No differences were noted between hemorrhage and LBNP. Statistical changes from baseline in pH, Po_2, Pco_2, BUN, and glucose were observed after both MAX hemorrhage and MAX LBNP.

Vasoactive hormone responses. At baseline, PRA, AVP, NE, and EPI were not different between hemorrhage and LBNP experiments (Fig. 5, *A–D*). MAX hemorrhage caused increases in PRA, AVP, NE, and EPI compared with baseline ($P < 0.01$). MAX LBNP elicited significant increases in PRA and EPI ($P < 0.01$), but not in AVP and NE.

DISCUSSION

The goal of the present study was to test the hypothesis that progressive hemorrhage and LBNP would elicit similar hemodynamic, neurohumoral, and metabolic responses when matched for CVP and pulse pressure. An experimental protocol designed to elicit similar hemodynamic responses at every step of LBNP and hemorrhage would be required to test this hypothesis. Since blood pressure changes minimally (7), and heart rate increases with significant variability (48) during the early stages of hemorrhage and LBNP, we dismissed these as potential matching variables. However, previous studies of CVP and pulse pressure during hemorrhage and LBNP revealed that these variables changed early and linearly during both procedures (20, 25, 59). CVP in humans has been shown to decrease linearly during blood loss to 450 ml (41, 44), and LBNP to −30 mmHg (26, 40, 59), but linear changes were not observed with greater levels of LBNP (59). On the other hand, pulse pressure correlated with stroke volume in humans during blood loss of 375 ml (33), and during LBNP to −60 mmHg (8, 13), but the linear relationship between pulse pressure and stroke volume was highly variable in individual subjects (13). Since

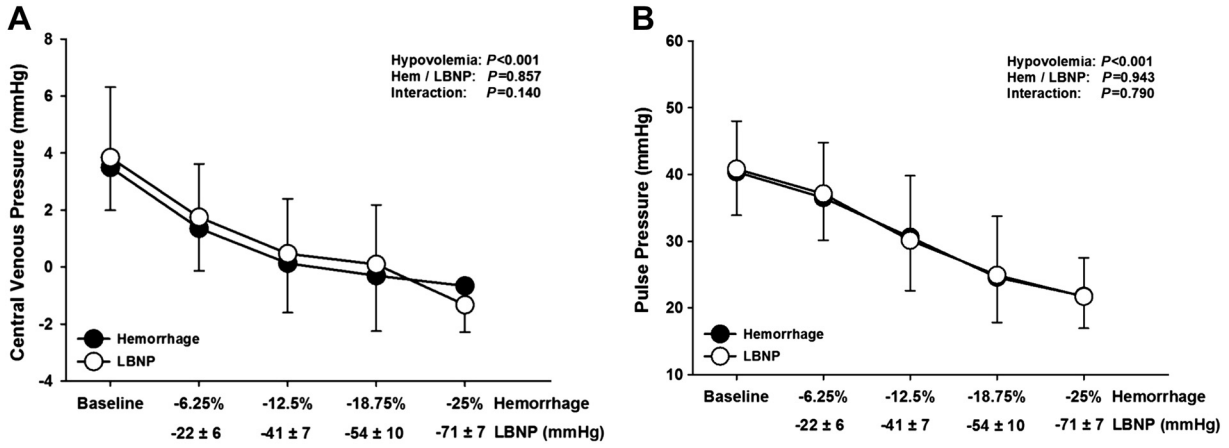

Fig. 1. Central venous pressure (*A*) and pulse pressure (*B*) during baseline and 4 steps of hemorrhage (●) and lower body negative pressure (LBNP; ○) corresponding to 6.25% ($n = 14$), 12.5% ($n = 14$), 18.75% ($n = 14$), and 25% ($n = 12$) total blood volume loss. Data are expressed as means ± SD. Two-factor repeated-measures ANOVA table *P* values are shown.

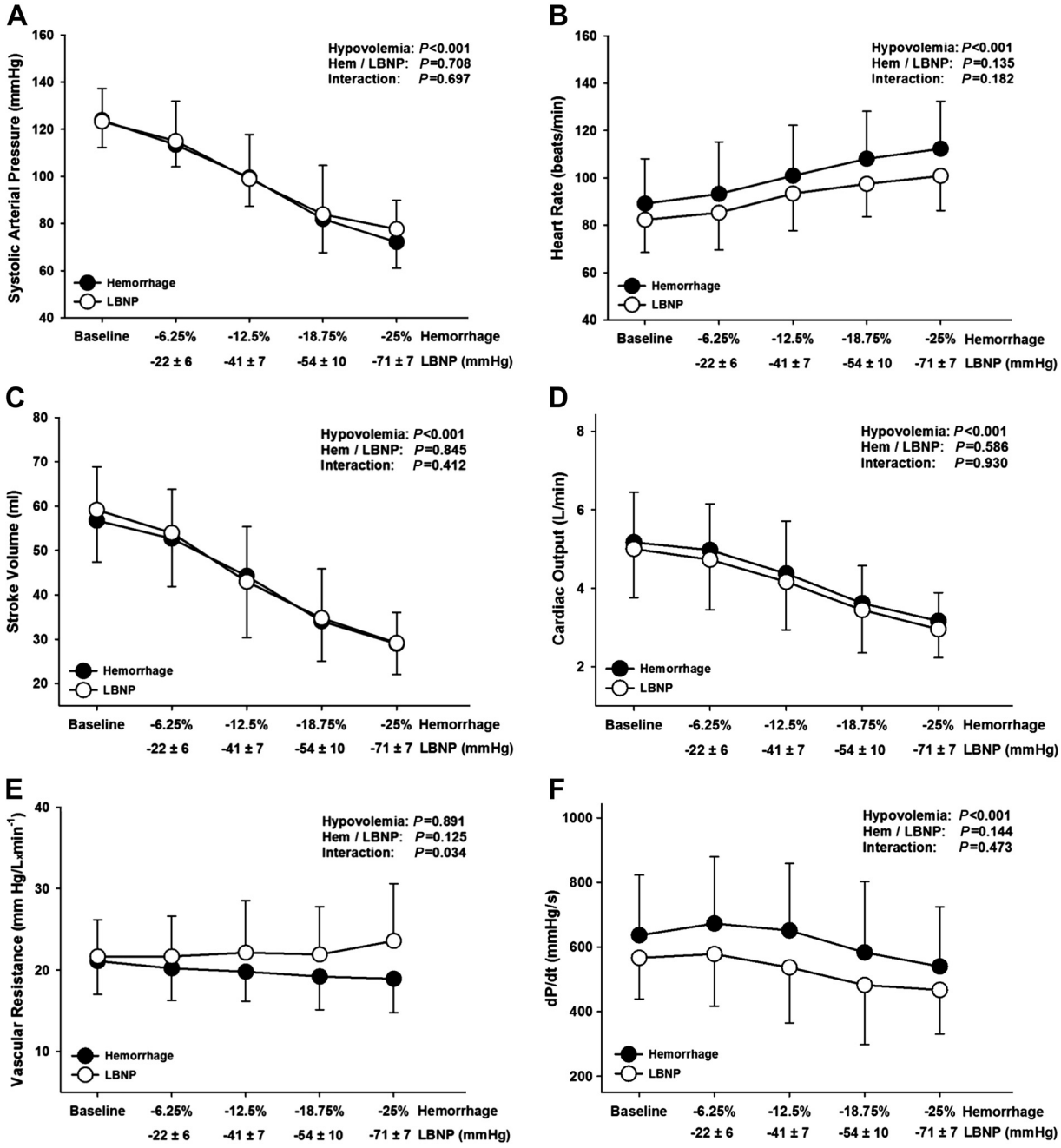

Fig. 2. Systolic blood pressure (*A*), heart rate (*B*), stroke volume (*C*), cardiac output (*D*), vascular resistance (*E*), and +dP/d*t* (*F*) during baseline and 4 steps of hemorrhage (●) and LBNP (○) corresponding to 6.25% ($n = 14$), 12.5% ($n = 14$), 18.75% ($n = 14$) and 25% ($n = 12$) total blood volume loss. Data are expressed as means ± SD. Two-factor repeated-measures ANOVA table *P* values are shown.

both CVP and pulse pressure changed early and linearly, and both have limitations as predictors of blood volume, we used both CVP and pulse pressure to match LBNP levels to hemorrhage steps in the same animal.

Heart and circulation. The current study revealed similar hemodynamic responses during both challenges of reduced central blood volume. While estimates of absolute levels of SV and cardiac output using the pulse contour method may be limited in accuracy resulting in higher cardiac output values than previously reported in baboons (36), our experimental design supports the notion that SV and cardiac output are identical between hemorrhage and LBNP experiments. In addition, responses in blood pressure, heart rate, SV, cardiac output, and +dP/d*t* were consistent with hemodynamic responses in other hemorrhage (2–3, 17, 29, 53) and LBNP studies (12, 31, 59). Interestingly, vascular resistance was stable during hemorrhage and LBNP in the present study, contrary to the increase in vascular resistance previously observed during hypovolemia (7, 9, 35, 53). Since studies in nonhuman primates sedated with only ketamine demonstrated increases in vascular resistance in response to LBNP (31), we speculate that the lack of a vasoconstrictor response may be

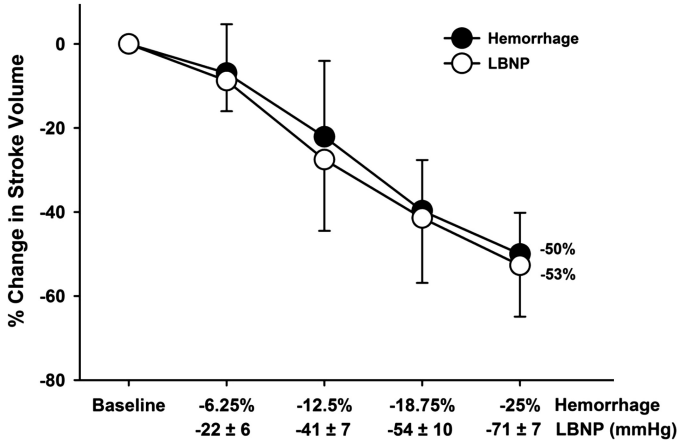

Fig. 3. Stroke volume expressed as percent change from baseline during 4 steps of hemorrhage (●) and LBNP (○) corresponding to 6.25% ($n = 14$), 12.5% ($n = 14$), 18.75% ($n = 14$) and 25% ($n = 12$) total blood volume loss. Data are expressed as means ± SD.

Table 2. *Metabolic responses to Hemorrhage and LBNP*

Variable	Study	BL	MAX
pH	Hem	7.27 ± 0.02	7.25 ± 0.03*
	LBNP	7.27 ± 0.04	7.25 ± 0.05*
P_{O_2}, mmHg	Hem	55.0 ± 7.0	35.9 ± 7.0*
	LBNP	53.8 ± 6.6	50.4 ± 9.2*
P_{CO_2}, mmHg	Hem	74.2 ± 4.0	79.0 ± 6.9*
	LBNP	75.3 ± 9.3	77.5 ± 9.1*
Na, mmol/l	Hem	146 ± 1	145 ± 2
	LBNP	147 ± 4	146 ± 3
Osm, osm/l	Hem	278 ± 2	279 ± 3
	LBNP	281 ± 7	281 ± 4
BUN, mg/dl	Hem	9.3 ± 2.3	10.5 ± 2.8*
	LBNP	10.3 ± 3.0	11.8 ± 3.2*
Glucose, mg/dl	Hem	67.6 ± 12.4	88.2 ± 25.1*
	LBNP	64.4 ± 7.08	80.0 ± 23.1*

Values are means ± SD; $n = 12$. Blood values of pH, oxygen partial pressure (P_{O_2}), carbon dioxide partial pressure (P_{CO_2}), sodium (Na), osmolarity (Osm), blood urea nitrogen (BUN), and glucose during hemorrhage (Hem) and LBNP at baseline (BL) and point of maximum Hem or LBNP (MAX) are shown. *Difference from baseline ($P < 0.01$). There were no differences between Hem and LBNP.

associated with the presence of valium used in this protocol to offset the stimulant effects of ketamine (28). Our experimental protocol of four equal progressive steps of relative total blood loss provided a unique opportunity to identify a linear relationship between total blood loss and SV (Fig. 3). Quantitatively, the results demonstrated a 2% reduction in SV for every 1% loss of total blood volume and reveals that 25% hemorrhage and corresponding LBNP level result in a 50% reduction in SV. Based on this stimulus-response relationship, the percent reduction in SV during hemorrhage can be estimated as twice the percent of blood loss. Therefore, it is not surprising that irreversible shock can occur with hemorrhage that results in

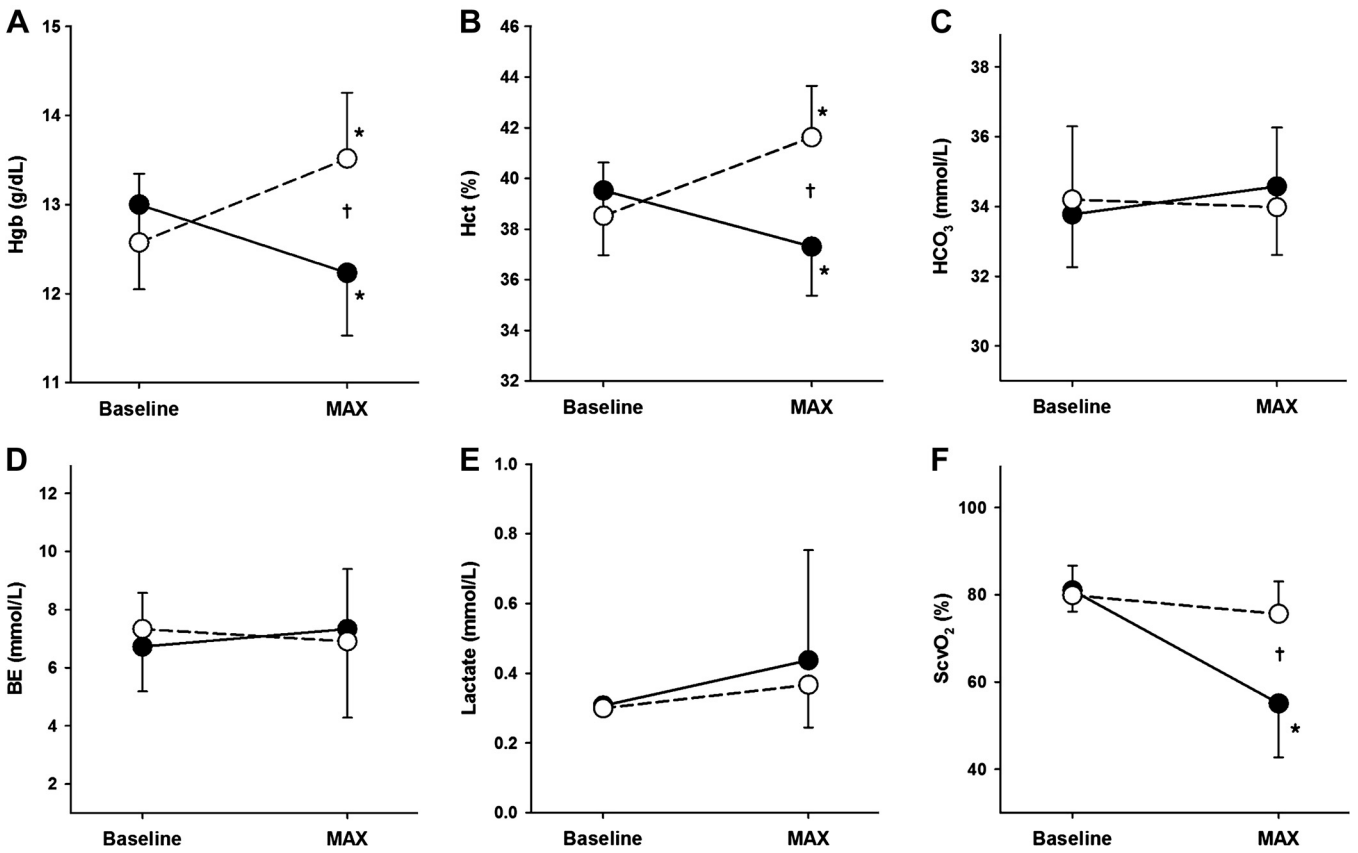

Fig. 4. Hemoglobin (Hgb, *A*), hematocrit (Hct, *B*), bicarbonate (HCO_3, *C*), base excess (BE, *D*), lactate (*E*), and central venous oxygen saturation ($ScvO_2$, *F*) are shown at baseline of hemorrhage (●, solid lines) and LBNP (○, dashed lines) and at the maximum level of hemorrhage and matching LBNP (MAX). Values are means ± SD; $n = 12$. *Difference from baseline ($P < 0.01$). †Difference between hemorrhage and LBNP ($P < 0.01$).

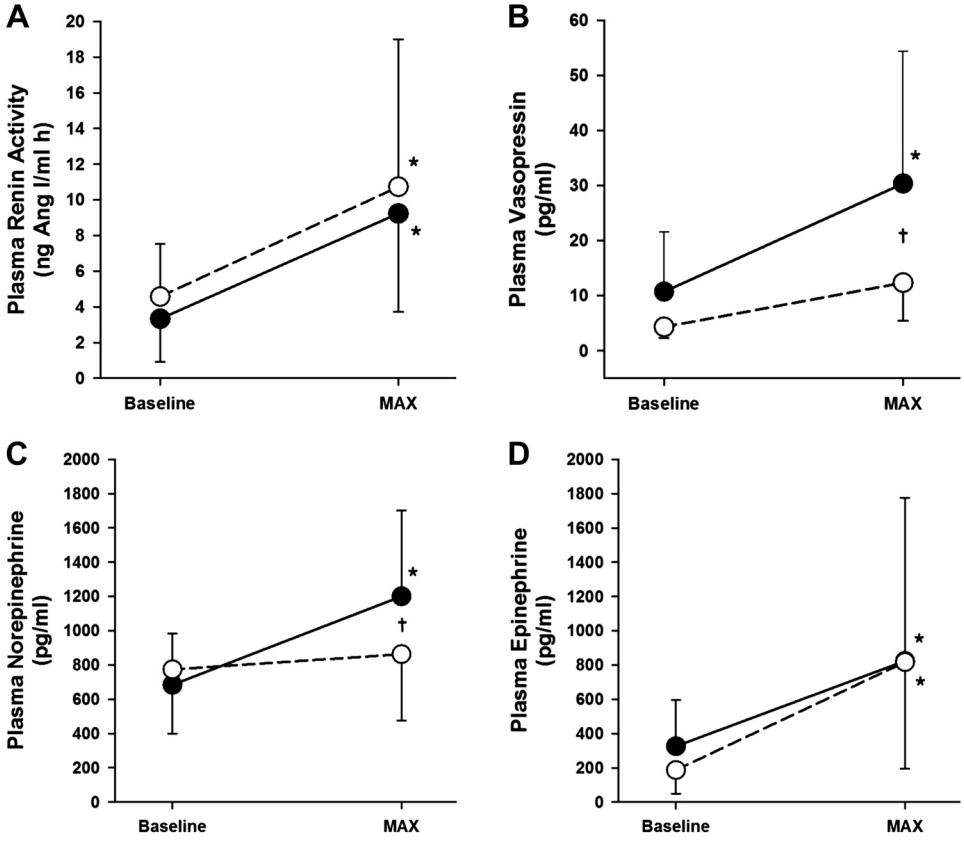

Fig. 5. Plasma renin activity (*A*), vasopressin (*B*), norepinephrine (*C*), and epinephrine (*D*) are shown at baseline of hemorrhage (●, solid lines) and matching LBNP (○, dashed lines), and at the maximum level of hemorrhage and matching LBNP (MAX). Values are means ± SD; $n = 12$. *Difference from baseline ($P < 0.01$). †Difference between hemorrhage and LBNP ($P < 0.01$).

>40% total blood loss (1) since this would translate to a reduction of >80% in SV.

Metabolic indexes of tissue dysoxia. A significant observation in this study was that hemorrhage and LBNP had opposite effects on Hgb and Hct (Fig. 4). Hemorrhage resulted in decreased Hgb and Hct, whereas LBNP produced a rise in both values. Decreases in Hgb and Hct during unresuscitated hemorrhage are well studied and are consistent with hemodilution resulting from the transfer of fluid from the extravascular to the intravascular compartment to compensate for blood volume loss (3, 30, 37). Increases in Hgb and Hct during LBNP have also been previously reported (14, 49, 63), and this difference from that observed with hemorrhage reflects the physiological mechanisms producing central hypovolemia. During LBNP, intravascular fluid shifts to the interstitial spaces of the lower extremities as a result of the pressure gradient and causes significant reductions in circulating plasma volume and observed tissue edema (60). Cellular components such as red blood cells are too large to traverse intact vessel walls, and the end result is an effective hemoconcentration due to the increased ratio of red cell mass to plasma within the intravascular space. Conversely, hemorrhage results in actual removal of red blood cell mass and possibly in compensatory autoresuscitation by movement of interstitial fluid back into the intravascular space in response to central hypovolemia.

Venous pH, HCO_3, BE, and lactate values did not differ between LBNP and hemorrhage, despite the differences observed with Hgb and Hct. Other investigators have reported onset of metabolic acidosis in more severe hemorrhage models (37, 54), but our findings indicate that hemorrhage to as much as 25% blood loss over the time course used in this study did not elicit this response. Similarly, previous reports of LBNP in humans found minimal changes in pH, lactate, and BE (63). Interestingly, we observed a difference in $ScvO_2$ at the MAX time point between the two studies with hemorrhage causing a significantly lower $ScvO_2$ than LBNP (55 ± 12% vs. 76 ± 7%, $P < 0.001$). We hypothesize that a lower $ScvO_2$ during hemorrhage can be explained by a reduced oxygen-carrying capacity or delivery (DO_2) due to less circulating Hgb (19), and may be responsible for the subtle divergence in vascular resistance during hemorrhage and LBNP (Fig. 2*E*). The contribution of Hgb to DO_2 in our hemorrhage model can be quantitatively assessed by calculating $DO_2 = [1.39 \times Hgb \times SaO_2 + (0.003 \times PaO_2)] \times Q$. Perhaps this highlights one subtle difference between hemorrhage and LBNP. The decrease in Hgb observed during hemorrhage may result in enough of a decrease in DO_2 to affect $ScvO_2$, but not enough to affect the global imbalance of the by-products of anaerobic metabolism to demonstrate a difference in BE or lactate.

Chemical analyte levels were similar between hemorrhage and LBNP (Table 2), and changes from baseline were observed in pH, P_{O_2}, P_{CO_2}, BUN, and glucose. Changes in these measures were minimal and likely to be clinically unimportant. However, it should be appreciated that significant changes in metabolites at the tissue level that reflect the onset of overt tissue dysoxia may not be reflected by measurements in the blood (63).

Neurohumoral activation. In our study, we evaluated the responses of PRA, AVP, NE, and EPI because of their vasoconstrictor actions during hemorrhage and LBNP. The re-

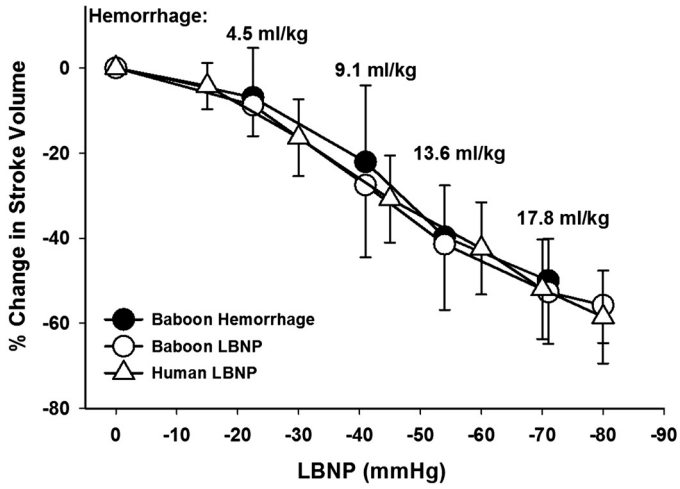

Fig. 6. Stroke volume expressed as percent change from baseline during 4 steps of hemorrhage in baboons (expressed as ml/kg, ●), the corresponding levels of LBNP in baboons (○), and during previous LBNP experiments in humans (△, $n = 117$). Data are expressed as means ± SD.

sponses in PRA and EPI were similar during hemorrhage and LBNP. The increases in PRA were consistent with previously reported activation of the renin-angiotensin-aldosterone system in response to hemorrhage (17, 52, 62) and LBNP (10, 49). Similarly, an increase in circulating EPI has been reported during hemorrhage (29, 38) and LBNP (14, 16, 22, 35) and is attributed to the selective activation of adrenal sympathetic nerves during the hypotensive stage of hypovolemia (61).

In contrast, AVP and NE levels increased during hemorrhage, but not during LBNP. While the increases in plasma AVP and NE levels during hemorrhage were similar to other studies (3, 29, 38, 58, 62), the lack of response during LBNP was unexpected considering previous reports from LBNP studies in humans. LBNP is associated with increases in plasma AVP levels, but the magnitude of AVP responses is variable. This variability in AVP response has been associated with the presence of presyncopal symptoms or the ability to tolerate high levels of LBNP (4, 10, 21). LBNP in humans has consistently resulted in activation of muscle sympathetic nerve activity (11, 27, 61, 64) and increases in circulating levels of NE (10, 14, 16, 22, 35). As such, the absence of a NE response in the present experiment appears to be protocol specific rather than the response generally reported.

We acknowledge that the comparison of our results to other studies is complicated by factors associated with species differences, methodological differences, and the effects of sedation and surgical procedures on baseline levels of PRA, AVP, NE, and EPI. However, it is unlikely that these factors are responsible for different responses between hemorrhage and LBNP since the conditions were similar during both hemorrhage and LBNP in our studies. Therefore, the lack of significant increases in AVP and NE during LBNP compared with hemorrhage indicates that hemorrhage was a stronger stimulus for AVP and NE release than LBNP in the present protocol, and suggests differences in the regulation of AVP and NE activation between the two forms of central hypovolemia induced in this study. We suspect that this difference may be associated with the proposed decrease in tissue oxygen delivery linked to the decrease in Hgb, Hct, and $ScvO_2$ during hemorrhage which was not observed during LBNP. Both LBNP and hemorrhage caused decreased perfusion, similar hemodynamic effects, and an increase of the stress response as exhibited by increases in PRA and EPI compared with baseline. However, the greater hypoxic stress may provide an added stimulus to activate AVP and NE during hemorrhage compared with LBNP. This speculation is supported by studies showing that acute hypoxia can increase AVP levels (18, 23, 46), muscle sympathetic nerve activity (34, 42, 56), and NE release at the neurovascular junction (34). Furthermore, a study by Raff et al. (43) reported an augmented increase in plasma AVP to hemorrhage during acute hypoxia compared with normoxic conditions.

Human blood loss equivalents of LBNP. Clearly, we have demonstrated that hemorrhage and LBNP elicit similar hemodynamic responses in nonhuman primates, but how do these responses compare to LBNP responses we have previously reported in humans? A direct comparison between baboon and human responses may be limited by the fact that the baboons were sedated with ketamine and valium, and humans were conscious. However, studies of the cardiovascular effects of ketamine and valium in humans indicate that sedation did not affect blood pressure, heart rate, and respiration (28, 39). Our data in baboons also reflect the lack of cardiovascular effects of ketamine and valium as all hemodynamic variables at baseline were within normal ranges. In addition, the tachycardia we observed during hypovolemia in the baboons indicated that the cardiac baroreflex was not abolished by sedation. Vascular resistance was the only hemodynamic variable that may have been affected by valium sedation in our study. In light of the minimal cardiovascular effects of sedation, we attempted to translate the baboon stroke volume responses to those of humans. We retrospectively analyzed the relative (percent) reduction in stroke volume during progressive LBNP from baseline to −70 mmHg extracted from our database of 117 human subjects and compared the human response to that of the baboons (Fig. 6). These comparisons demonstrate a striking similarity between humans and baboons in the responses to hemorrhage and LBNP even though the rate of hemorrhage and LBNP application was slower in baboons (7 min/step) than in humans (5 min/step). Further, the translation of the human LBNP response to hemorrhage is supported by comparisons of

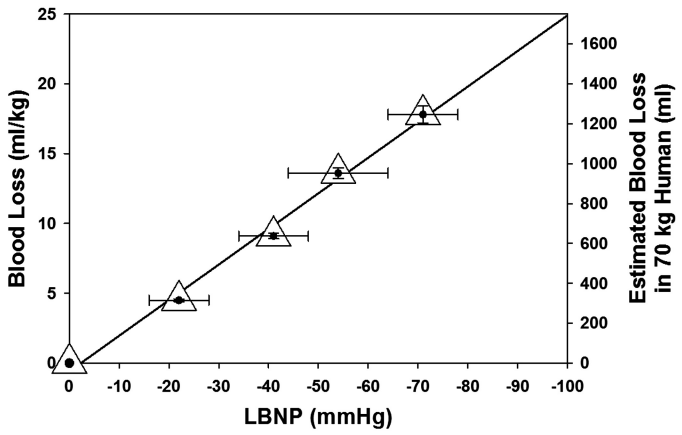

Fig. 7. Estimates of blood loss in humans (△, ml) corresponding to various levels of LBNP calculated from the results from the current study in baboons (ml/kg, ●). Data are expressed as means ± SD.

calculated blood volumes. The bleed applied to the baboons resulted in an average 18 ml/kg total blood volume reduction. Since the total blood volume of average fit human males is ~75 ml/kg (6), the translation of our results to humans is supported by the extrapolation of an 18 ml/kg blood loss representing ~24% loss of total circulating blood compared with the 25% bleed used in our baboon experiment.

Another similarity of our results in baboons to those observed in humans is tolerance to hypovolemia. We have observed that approximately one-third of subjects exposed to LBNP have a low tolerance to hypovolemia which is defined as the inability to complete −60 mmHg LBNP (10). In the current study, two baboons attained the termination criterion at the third step of hemorrhage (18.75% blood loss). Their limited compensatory response to blood loss was also observed when these animals were subsequently exposed to LBNP and the protocol was terminated at −50 mmHg in one animal and −55 mmHg in the other animal. This observation provides further support that the compensatory responses to LBNP reflect those observed during hemorrhage and that baboons are similar to humans in these responses.

Despite the extensive use of LBNP as an experimental model of hemorrhage, the magnitude of blood loss that equates to the magnitude of LBNP has not been adequately compared in the human subjects. Two studies have compared the hemodynamic responses to 450 ml blood donation to those observed during low levels of LBNP. Comparisons in the same subjects revealed that 450 ml blood loss was equivalent to −10 mmHg LBNP (44), while comparisons in separate groups of subjects showed that 450 ml blood loss was equivalent to −20 mmHg LBNP (24). Cooke et al. (12) estimated ranges of blood loss volumes for several ranges of LBNP levels by using cross-sectional comparisons of physiological responses acquired over 40 years from LBNP studies to responses observed during actual hemorrhage studies in animals and humans. They predicted that the responses to mild hemorrhage (~10% total blood volume), moderate hemorrhage (10–20% total blood volume) and more severe hemorrhage (greater than 20% blood volume loss) would be similar to −10 to −20, −20 to −40, and greater than −40 mmHg LBNP, respectively. Most recently, Summers et al. (57) used a computational model of human physiology and simulated a LBNP procedure on a virtual subject (70 kg) to predict the amount of blood displaced by LBNP. In this virtual environment, LBNP of −15, −30, and −60 mmHg displaced 486, 664, and 938 ml of blood.

Using the relative blood loss volumes (ml/kg) calculated from the current investigation, we determined the absolute blood volume loss in a 70 kg human which equate to the levels of LBNP observed in our baboons (Fig. 7). The linear relationship between LBNP and blood loss provides a metric to estimate the magnitude of blood loss over a broad range of LBNP. Based on this metric, −30, −60, and −90 mmHg LBNP is equivalent to 450, 1,000, and 1,600 ml blood loss in a human with 70 kg body wt. This comparison represents the first time that a wide range of LBNP pressures can be translated to hemorrhage volume in humans. Overall, this study provides valuable insight into the similarities and differences between experimental hemorrhage and LBNP and suggests that actual loss of blood cells during hemorrhage may result in greater activation of some compensatory mechanisms compared with LBNP. Interestingly, and most importantly, these differences did not affect the integrated hemodynamic profile of hemorrhage and LBNP. Therefore, this study provides three significantly novel results. First, the hemodynamic responses to hemorrhage and LBNP in nonhuman primates are equivalent such that progressive increases in LBNP to as much as −70 mmHg can be used to define the hemodynamic response to hemorrhage up to 25% loss of total blood volume. Second, a linear relationship between SV and blood loss demonstrates a 2% reduction in SV for every 1% loss of total blood volume. Third, the hemodynamic responses to LBNP are similar in baboons and humans, which allows for a metric to predict the magnitude of blood loss during progressive LBNP in humans. Overall, this study provides compelling inferential support of the validity of LBNP as a physiological simulation of hemorrhage in humans.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: C.H.-L., R.E.S., G.W.M., C.B., H.F.P., A.P.C., and V.A.C. conception and design of research; C.H.-L., R.E.S., G.W.M., C.B., H.F.P., and V.A.C. performed experiments; C.H.-L., R.E.S., K.A.G., H.F.P., and V.A.C. analyzed data; C.H.-L., R.E.S., G.W.M., C.B., K.A.G., H.F.P., K.K.C., A.P.C., and V.A.C. interpreted results of experiments; C.H.-L. prepared figures; C.H.-L. drafted manuscript; C.H.-L., K.A.G., H.F.P., K.K.C., A.P.C., and V.A.C. edited and revised manuscript; C.H.-L., R.E.S., G.W.M., C.B., K.A.G., H.F.P., K.K.C., A.P.C., and V.A.C. approved final version of manuscript.

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